SUMMARY OF SAFETY AND EFFECTIVENESS DATA

1. General Information

1.1. Device Generic Name: Immunoassay for hepatitis B surface antigen

1.2. Device Trade Name: ETI-MAK-2 PLUS

1.3. Applicant's Name and Address:

DiaSorin
Via Crescentino
Saluggia (VC) 13040, Italy

1.4. U.S. Representative:

Sienna Partners, LLC
P.O. Box 103
Baldwin, MD 21013

1.5. PMA Number: P990038

1.6. Date of Panel Recommendation: January 20, 2000

1.7. Date of Notice of Approval to the Applicant: March 30, 2001

2. Indications For Use

ETI-MAK-2 PLUS is an in vitro enzyme immunoassay (EIA) intended for use in the qualitative determination of hepatitis B surface antigen (HBsAg) in human serum or plasma (EDTA, citrate or heparin). The ETI-MAK-2 PLUS is intended for manual use and with the Biochem Immunosystems Labotech/ETI-LAB automated instrument.

The detection of HBsAg is indicative of a laboratory diagnosis for hepatitis B virus (HBV) infection, either acute or chronic. Further HBV serological marker testing is required to define the specific disease state. The ETI-MAK-2 PLUS assay's performance has not been established for the monitoring of HBV disease or therapy.

3. Device Description

3.1. Principle of The Assay

ETI-MAK-2 PLUS uses monoclonal antibodies to hepatitis B surface antigen (HBsAg) as the basis for this enzyme immunoassay. The assay is a direct, non-competitive test based on the use of polystyrene microwells coated with mouse monoclonal antibodies to HBsAg (directed to the "a" determinant of HBsAg). An enzyme tracer containing horseradish

peroxidase-labeled sheep antibodies to HBsAg detects any captured HBsAg from the patient's specimen.

In the assay procedure, patient specimens and controls are incubated with incubation buffer in anti-HBs-coated microwells. If HBsAg is present in a specimen or control, it binds to the antibodies. Excess sample is removed by a wash step, and the enzyme tracer is then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. Excess enzyme tracer is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains HBsAg, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue color (650 nm). The blue color turns to yellow (450 nm) after addition of the stop solution. If a sample does not contain HBsAg, the microwell will be colorless after the chromogen/substrate solution is added and will remain colorless after the stop solution is added. Color intensity, which is measured spectrophotometrically, is indicative of the concentration of HBsAg. Absorbance value readings for patient specimens are compared to a cutoff value determined from the mean of the calibrators.

3.2. Kit Configuration and Components

For detection of HBsAg, the ETI-MAK-2 PLUS system is comprised of the following:

Coated Strips
Microwells coated with mouse monoclonal antibodies to HBsAg (IgG1-k class, directed to the "a" determinant of HBsAg).

Enzyme Tracer
Horseradish peroxidase-labeled sheep IgG antibodies to HBsAg, buffer, protein stabilizers.
Preservative: 0.2% ProClin 300.

Tracer Diluent
Buffer, human serum/plasma, protein stabilizers.
Preservative: 0.2% ProClin 300.

Calibrator (Human)
Human serum/plasma non-reactive for HBsAg.
Preservative: 0.2% ProClin 300.

Negative Control (Human)
Human serum/plasma non-reactive for HBsAg.
Preservative: 0.2% ProClin 300.

Positive Control (Human)
Human serum/plasma reactive for HBsAg (subtypes ad and ay), protein stabilizers.
Preservative: 0.2% ProClin 300.

Incubation Buffer
Buffer, protein stabilizers, an inert blue dye.
Preservative: 0.2% ProClin 300.

Wash Buffer (concentrate)
Buffer, detergents, preservatives.

Chromogen/Substrate

Tetramethylbenzidine/hydrogen peroxide system.

Stop Solution
1N sulfuric acid

Strip Sealers

Plate Sealers

Pouch Sealer

4. Contraindications

None

5. Warnings and Precautions

For in vitro diagnostic use only.

Warnings and precautions for users of the ETI-MAK-2 PLUS assay are stated in the product labeling.

6. Alternative Practices and Procedures

Historically, the presence of HBsAg, the main HBV serologic marker was determined using the immunodiffusion method. Since the method was very specific but with low sensitivity, other techniques such as counterimmunoelectrophoresis and complement fixation were developed. The advent of the newer technologies of complement fixation, counter immunoelectrophresis, reversed passive hemagglutination, inhibition of passive hemagglutination for subtyping, RIA and enzyme-linked immunosorbent assay (ELISA) has yielded a further improvement of assay sensitivity. Currently, the commercially available kits are based on RIA or ELISA techniques that are similar in sensitivity and specificity.

7. Marketing History

The ETI-MAK-2 PLUS assay has never been marketed in the US or outside the US.

8. Potential Adverse Effects of the Device on Health

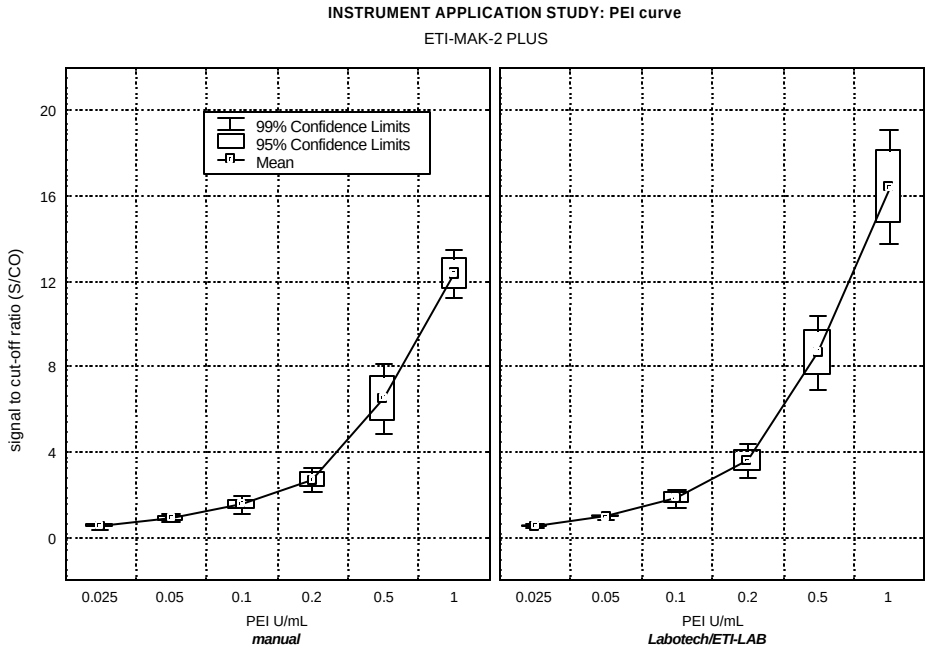
Failure of the product to perform as indicated or human error in use of the product may lead to a false result. A false negative result may be considered a patient or public health concern because the patient may be considered in early recovery and proceeding to HBV clearance. In this case, the risk may be that the patient's treatment may be altered or that they might not be considered infectious. This could lead to possible infection of uninfected individuals. A false positive result cannot be considered a patient or public health concern because the disease-state interpretation would be active HBV infection. In a false positive test, if HBsAg is the only hepatitis B serological marker detected, other additional testing should be done to validate the results e.g., testing new patient specimens or testing with an HBsAg confirmatory assay. This additional testing would invalidate the initial false positive test.

9. Summary of Preclinical Studies

9.1. Comparison of Labotech Instrumentation with the Manual Assay

An instrument application study was conducted at DiaSorin, Saluggia Italy, to evaluate the performance of the ETI-MAK-2 PLUS assay on the Biochem Immunosystems Labotech/ETI-LAB, an automated microplate processing instrument, compared to the manual analysis. The Paul-Ehrlich-Institut (PEI) Standard, 12 serum samples near the ETI-MAK-2 PLUS cutoff and samples from the clinical trials (32 suspected hepatitis B patients and 8 apparently healthy adults) were tested in parallel manually and on the Labotech.

Serial dilutions of the PEI Standard were prepared in fetal calf serum to obtain a panel ranging from high concentration to below the analytical sensitivity of the assay. The diluted Standard samples were tested in duplicate, one run per day for three days both manually and on the Labotech. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The 95% confidence intervals were established for the S/CO values of each point of the Standard-referenced curve and therefore the analytical endpoint sensitivity was defined (first dilution with S/CO > 1.1). A graph summarizing these results is presented below:



The 12 samples near the cutoff were tested in triplicate, one run per day for three days both manually and on the Labotech. The samples from the clinical trials were tested in singlet in one run on one day, both manually and on Labotech. The mean, the standard deviation and the coefficient of variation (CV%) of the S/CO values were computed by the different components of variability for each of the tested specimens. A summary of the data is presented in the following table.

	Manual			Labotech/ETI-LAB		
Analytical Endpoint Sensitivity (0.1 PEI U/mL)	Mean	W/R %CV[a]	D/D %CV	Mean	W/R %CV	D/D %CV
S/CO [95% CI][b]	1.56 [1.29 – 1.82]	11.8	15.1	1.84 [1.56 – 2.12]	4.1	15.7
12 Cutoff Samples S/CO	1.21	11.2	7.9	1.43	4.0	13.0
Range of mean S/CO	0.91 – 1.44			1.11 – 1.80		
Clinical Samples:						
Suspected Hepatitis B Range of S/CO	Negative: N/A (0/32) Equivocal: N/A (0/32) Positive: 23.9 – >39 (32/32)			Negative: N/A (0/32) Equivocal: N/A (0/32) Positive: 35.5 – >62 (32/32)		
Healthy Adults Range of S/CO	Negative: 0.24 – 0.64 (8/8) Equivocal: N/A (0/8) Positive: N/A (0/8)			Negative: 0.08 – 0.46 (8/8) Equivocal: N/A (0/8) Positive: N/A (0/8)		

[a] %CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate
[b] 95% CI = 95% Confidence Interval; W/R = within-run; D/D = day-to-day

No reproducibility testing with the Labotech instrument was conducted. As part of the conditions of approval agreement, DiaSorin will provide FDA results from a reproducibility study using the Labotech instrument. Until that condition is met, the following statement will be placed in the labeling:

> "Assay reproducibility using the Labotech has not been established. If the Labotech is used, the user should establish appropriate assay reproducibility in accordance with NCCLS EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices."

9.2. Analytical Sensitivity

The analytical sensitivity of the assay (the smallest quantity of analyte that can be distinguished from background) was evaluated using single point serial dilutions of a standard preparation from the Paul-Ehrlich-Institut (PEI). The analytical sensitivity of the assay (last positive dilution) was determined to be 0.1 PEI U/mL (Mean Signal-to-Cutoff Ratio = 1.56; 95% Confidence Interval = 1.29 to 1.82).

9.3. Potential Cross-Reacting Substances

Serum samples were obtained from patients belonging to a number of different disease catergories listed below. Of the 525 potential interfering samples, 477 (91%) were negative and 48 (9%) were positive by ETI-MAK-2 PLUS. Among the 48 positive samples, 12 were negative for HBeAg and IgM anti-HBc and negative for HBsAg on repeat testing; 36 were positive by reference testing or review of hepatitis B marker patterns for those samples. As expected, individuals infected by HDV are also infected with HBV. Disease was determined by serological testing, there is no guarantee that the associated antigen was present in the tested material.

Cross-Reactivity Study Results

GROUP	N	ETI-MAK-2 PLUS Negative or Equivocal Samples	ETI-MAK-2 PLUS Positive Samples	% Confirmed Positive By Additional Testing
Acute EBV infection	16	13	3[a]	–
Acute CMV infection	20	17	3[a]	–
Acute HSV infection	10	10	0	–
Acute Toxoplasma infection	18	18	0	–
Acute parvovirus B19 infection	5	5	0	–
HTLV-I/II infection	50	47	3[b]	100% (1/1)
Syphilis	26	25	1[a]	–
HCV infection	50	48	2[a]	–
HDV infection	20	1	19	100% (19/19)
HIV infection	50	50	0	–
Acute HAV infection	50	47	3	100% (3/3)
Past HAV infection	50	44	6[c]	100% (5/5)
Rheumatoid factor (RF) +	40	40	0	–
Autoimmune disease, including SLE	30	30	0	–
Autoimmune hepatitis	5	5	0	–
Myeloma	20	20[d]	0	–
Hypergammaglobulinemia	20	20	0	–
Influenza vaccine	5	5	0	–
Elevated liver enzymes	10	9	1	100% (1/1)
Non-viral liver disease	30	23	7	100% (7/7)
TOTAL	525	477 (91%)	48 (9%)	100% (36/36)

[a] These samples were negative for HBeAg and IgM anti-HBc, and negative on repeat testing on ETI-MAK-2 PLUS.

[b] 2 samples were negative for HBeAg and IgM anti-HBc, and negative on repeat testing on ETI-MAK-2 PLUS.

[c] 1 sample was negative for HBeAg and IgM anti-HBc, and negative on repeat testing on ETI-MAK-2 PLUS; 5 samples were positive by reference testing.

[d] 1 sample was repeatedly equivocal and all other markers were negative.

A BLAST analysis [Basic Local Alignment Search Tool, National Center for Biotechnology Information, National Institutes of Health, http://www.ncbi.nlm.nih.gov/BLAST] was performed to determine if untested viral or bacterial proteins could potentially cross-react with the anti-HBs monoclonal antibodies used in this assay. No notable similarities in protein sequences were identified from these viral or bacterial proteins, suggesting that they should not cross-react in this assay.

9.4. Interfering Substances

The ETI-MAK-2 PLUS assay was evaluated for interference by testing the substances identified in the table below. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with high-titer HBsAg samples to obtain a result near the cutoff. None of the compounds at the levels indicated were found to interfere with the clinical interpretation of the assay in serum. No interference was found with bilirubin in plasma (EDTA, heparin or citrate), testing for interference with hemoglobin and triolein was not performed in plasma.

Compound	Concentration	
Bilirubin	0.18 mmol/L	10 mg/dL
Hemoglobin	0.06 mmol/L	100 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

The ETI-MAK-2 PLUS assay was also evaluated for possible interference from heterophilic anti-mouse antibodies (HAMA). A dilutional panel was used, consisting of 21 samples prepared from a stock pool of high positive human serum. The HAMA concentrations in the samples ranged from 0 to 2975.5 ng/mL, as determined by a HAMA ELISA. In a direct non-competitive assay, such as ETI-MAK-2 PLUS, interference would manifest as false negative results. No interference was seen in that all 21 dilutions were negative by the ETI-MAK-2 PLUS assay, including the HAMA negative panel member.

9.5. Stability Studies

9.5.1. Kit Stability

Stability studies were performed on 3 different ETI-MAK-2 PLUS kit lots. At specified intervals from time of kit release, performance of the kits was evaluated by testing the Calibrator, Negative and Positive Controls, HBsAg calibration curve (subtype ad and ay; Paul Ehrlich Institut [PEI] referenced), and Q.C. sera panel according to the instructions for use. The kit must meet established acceptance criteria. The obtained stability data demonstrate that the kit performance is acceptable for at least 6 months. On the basis of the stability results, a shelf life of 6 months has been established for the kit.

9.5.2. Working Enzyme Tracer Stability

The Enzyme Tracer was diluted with the Tracer Diluent to obtain the working Enzyme Tracer according to the instructions for use. After 7 days from dilution, the performance of the kit was evaluated, according to the instructions for use, testing various specimens with freshly prepared working Enzyme Tracer and the 7-days old working Enzyme Tracer. The kit must meet established acceptance criteria. The tests on the working Enzyme Tracer demonstrate that the performance of the kit is acceptable when the 7 day-diluted Enzyme Tracer is used. The working Enzyme Tracer can be used for one week if stored at 2-8 °C.

9.5.3. Working Wash Buffer Stability

The Wash Buffer concentrate was diluted with deionized water according to the instruction for use to obtain the working Wash buffer. After 7 days from dilution, the performance of the kit was evaluated by testing various specimens with a freshly prepared working Wash buffer and the 7-days-old working Wash buffer, according to the instructions for use. The kit must meet established acceptance criteria. The tests on the working Wash Buffer demonstrate that the performance of the kit is acceptable when the 7 day-old working Wash Buffer is used. The working Wash Buffer can be used for one week if stored at 2-8 °C.

9.6. Common Reagents interchangeability Study

Studies were performed to demonstrate that the lots of some components included with ETI-MAK-2 PLUS kit and common to all kits of ETI-PLUS line (Wash Buffer, Chromogen/Substrate, Stop Solution), can be exchanged with other lots of the same component produced for the ETI-PLUS line (interchangeablity). Three lots of Wash Buffer, Chromogen/Substrate and Stop Solution were combined with one lot of ETI-MAK-2 PLUS; the three combinations were then tested with various samples. Regression analysis was applied to the Optical Density's of the samples. The regression analyses for the three studies gave slopes close to 1.0, with low intercepts and excellent correlation values. These results indicate that the use of different lots of Wash Buffer, Chromogen/Substrate and Stop Solution with the same ETI-MAK-2 PLUS lot gave equivalent results with samples distributed over the range of reactivity, confirming their interchangeablity.

9.7. Reproducibility

Manual Assay: Intra-assay, inter-assay, inter-lot, and inter-site variability studies were carried out on the ETI-MAK-2 PLUS kit to test the variability within runs, between runs, between days, between kit lots, and between test sites. Variability was measured on a panel of ten sera that included negative, borderline, and positive samples. Three ETI-MAK-2 PLUS kit lots were tested at three independent test sites. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance to cutoff ratios (S/CO) rather than absolute absorbance values. The results of that study are tabulated below showing the assay reproducibility to be satisfactory.

Clinical Site Reproducibility Study

ID#		# of Tests per Sample	Mean S/CO's	Within-run %CV*	Between-runs %CV	Between-lots %CV	Between-days %CV	Between-sites %CV	Total
S01	High	108	13.09	6.08	12.11	10.63	9.70	9.03	16.51
S02	High	108	5.59	4.44	10.89	7.82	11.60	8.98	17.50
S03	Low	108	3.19	4.51	11.45	12.04	7.76	9.33	18.66
S04	Low	108	2.70	7.47	8.14	12.65	8.61	6.72	20.18
S05	Low	108	3.67	7.81	8.17	7.24	9.71	8.04	14.83
S06	Low	108	2.57	4.40	17.13	12.58	16.81	7.31	25.88
S07	Equiv	108	1.33	5.37	13.09	10.89	14.11	15.62	29.05
S08	Equiv	108	0.96	9.19	10.09	13.48	8.82	5.00	24.04
S09	Equiv	108	1.01	15.66	13.89	16.93	20.65	3.66	32.00
S10	Neg	108	0.22	15.71	29.33	30.67	59.12	21.18	104.13

* %CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate

9.8. Plasma Reproducibility

A plasma reproducibility study was conducted at DiaSorin, Saluggia Italy, to evaluate the performance of the manual ETI-MAK-2 PLUS assay on serum versus a variety of plasma types. The plasma types evaluated were citrate, heparin and EDTA. Sample sets of matched serum/multiple plasma were used in the study. A sample set was prepared by spiking the same high-positive sample into each of the matrices (serum and plasmas) resulting in a total of four specimens per set around the cutoff. Several high-positive samples were used in the preparation of the 12 different near-cutoff sample sets. Six matched serum/multiple plasma samples sets were tested in triplicate in each run; thus there were two runs per day for three days, all tested in a manual mode. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance to cutoff ratios (S/CO) rather than absolute absorbance values. The mean, the standard deviation and the coefficient of variation (CV%) of the S/CO values were computed by the different components of variability for each of the tested specimens. The 95% confidence intervals were established for the S/CO values of all serum samples and each plasma type. A summary of the data is presented in the following table. The study supports the use of plasma specimens in the ETI-MAK-2 PLUS assay.

	Serum	Citrate	Heparin	EDTA
Mean S/CO	0.91	0.92	0.87	0.91
95% CI*	[0.87-0.94]	[0.88-0.96]	[0.83-0.91]	[0.88-0.95]
W/R %CV**	6.3%	6.9%	7.1%	7.8%
D/D %CV	8.1%	9.6%	6.7%	8.0%
Total %CV	9.9%	11.6%	9.6%	10.0%
Between matrix %CV: 8.5%				
Across matrix total %CV: 13.1%				

* 95% CI = 95% Confidence Interval; W/R = within-run; D/D = day-to-day
** %CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate

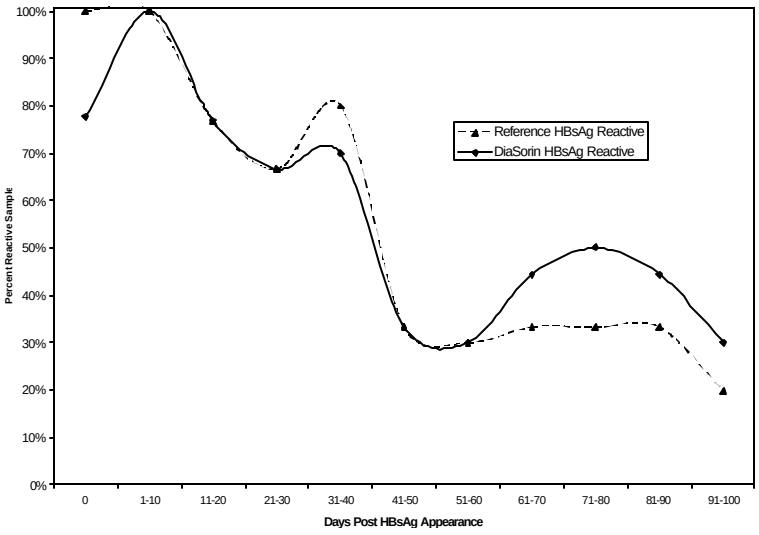
9.9. Acute Serial Seroconversion Panels

One hundred twenty-four (124) archived serial samples from nine individuals were tested for the appearance of HBsAg. Most (8/9) of these individuals were defined as being acutely infected by the appearance of HBsAg and HBeAg with the subsequent appearance of IgM anti-HBc, total anti-HBc, anti- HBe, and anti-HBs. One individual had detectable HBsAg, but did not have detectable HBeAg in any specimen. However, this individual did seroconvert for anti-HBe.

The specimens were collected from individuals undergoing plasmapheresis for further manufacturing purposes. Three individuals were found to be infected with HBV during the first plasmapheresis and others became infected with HBV during subsequent plasmapheresis. It is unknown how long these three initially HBsAg reactives were infected prior to the first plasmapheresis. All nine individuals underwent sequential plasmapheresis after becoming HBV infected. However, the timing of subsequent plasmapheresis varied from individual to individual. The specimens draw times were normalized to represent the day that HBsAg was first detected by an FDA-licensed assay as day 0. Draw days ranged from day 0 (HBsAg first detected) through day 355 post-day 0. Since all panels did not contain the same draw day, sample results were grouped within day intervals (e.g., days 0, 1-10, 11-20, etc., representing days since first detection of HBsAg).

The results are summarized in the following table and graph. In the graph below the pattern for the reference HBsAg percent reactive has been overlaid for reference. The reference assay and the ETI-MAK-2 PLUS assay were very similar in performance when detecting HBsAg in seroconversion panels.

Day Range	Number Specimens	DiaSorin HBsAg Reactive	% Positive
0	9	7	77.8%
1-10	10	10	100.0%
11-20	13	10	76.9%
21-30	9	6	66.7%
31-40	10	7	70.0%
41-50	6	2	33.3%
51-60	10	3	30.0%
61-70	9	4	44.4%
71-80	6	3	50.0%
81-90	9	4	44.4%
91-100	10	3	30.0%
101-110	6	1	16.7%
111-120	4	1	25.0%
121-130	4	1	25.0%
131-140	3	0	0.0%
141-150	2	1	50.0%
151-160	0	0	NA
161-170	1	0	0.0%
171-180	0	0	NA
181-190	1	0	0.0%
191-200	1	0	0.0%
355	1	0	0.0%



9.10. High Risk Population

Single repository samples belonging to high-risk populations (66 hemodialyzed patients, 148 hemophiliacs, 150 IV drug users) were tested with the DiaSorin ETI-MAK-2 PLUS assay to determine frequency of positive results in that population. The group was 12% (42/364) female, 69% (252/364) male, and 19% (70/364) unspecified, with ages ranging from 19 to 87 years old. No geographical locations were specified. The table below summarizes the ETI-MAK-2 PLUS results. The data in the table represent the number of specimens and prevalence rate in each high risk population.

High Risk Population

Population	Frequency of Positive Results (# Positive/Total # Samples)
IV drug users	17/150 = 11.3% (2 equivocal)
Hemophiliacs	12/148 = 8.1%
Hemodialysis patients	0/66 = 0.0%
TOTAL	29/364 = 8.0% (2 equivocal)

9.11. Expected Values Study

The 236 prospective patient samples used in the expected values study for the ETI-MAK-2 PLUS assay to detect HbsAg were from patients who were sent to the laboratory for HBV testing. Of those, 100 (42%) were frozen and 136 (58%) were fresh. The patients represented Florida, Georgia, Pennsylvania, California, Utah, and the southeastern US. The group was 69% (162/236) female, 29% (68/236) male, and 2% (6/236) unspecified; the ethnicity of the patients was unspecified. The ages ranged from 5 to 88 years old, with 6 samples unspecified. The prevalence rate for HBsAg in patients who were sent to the laboratory for HBV testing was 10%.

The table below summarizes the percent ETI-MAK-2 PLUS positive and negative results by gender and age range. There were 6 samples for which gender and age were not reported; they were all positive. There were 6 other samples for which age was not reported, two were from females and four were from males; all were negative. These 12 results were not included in the table.

Expected Values Summary

		DiaSorin ETI-MAK-2 PLUS						
		+		-		E*		TOTAL
Age Range	Gender	n	%	N	%	n	%	
0-9	F	0	0	2	100	0	0	2
	M	0	0	0	0	0	0	0
10-19	F	1	6	16	94	0	0	17
	M	1	50	1	50	0	0	2
20-29	F	3	6	48	94	0	0	51
	M	4	31	9	69	0	0	13
30-39	F	1	2	48	98	0	0	49
	M	3	18	14	82	0	0	17
40-49	F	3	15	16	80	1	5	20
	M	2	14	12	86	0	0	14
50-59	F	1	20	4	80	0	0	5
	M	1	13	7	88	0	0	8
60-69	F	1	33	2	67	0	0	3
	M	0	0	2	100	0	0	2
70-79	F	1	10	9	90	0	0	10
	M	0	0	5	100	0	0	5
80-89	F	1	33	2	67	0	0	3
	M	0	0	3	100	0	0	3
TOTAL		23	10	200	89	1	1	224

*E = equivocal result

10. Summary of Clinical Studies

10.1. Clinical Sample Testing

10.1.1. Prospective Samples

A study of 136 prospective specimens was conducted. These specimens represented individuals who were sent to the laboratory for hepatitis testing. Specimens were collected at a reference laboratory and assayed at the California clinical trial site. The patients were 86% (117/136) female and 14% (19/136) male. The ages ranged from 5 to 77 years old, with three specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-MAK-2 PLUS procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved labeling, including confirmation by neutralization of repeatably reactive HBsAg samples.

Results by Specimen Classification

After study completion all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were five unique HBV marker patterns observed in the ETI-MAK-2 PLUS prospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HbeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Chronic Infection	+	-	-	+	+	-	1
Recovery	-	-	-	+	+	+	2
Past Infection	-	-	-	+	-	+	4
	-	-	-	+	-	-	4
HBV Vaccine Response	-	-	-	-	-	+	38
Not Previously Infected with HBV	-	-	-	-	-	-	87

Based on the above classifications the ETI-MAK-2 PLUS HBsAg results for the prospective samples were compared to a reference assay's HBsAg results. The following table shows this comparison and percent agreement with 95% confidence intervals with the reference HBs results.

Prospective Samples Comparison

	Reference HBsAg[a]			TOTAL
	–		+	
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS	
Serological Classification	–	E[b]	+	
Chronic infection	0	0	1	1
Recovery	2	0	0	2
Past infection	8	0	0	8
HBV vaccine response	38	0	0	38
Not previously infected	86	1	0	87
Grand Total	134	1	1	136

[a] Result of initially repeatedly reactive and neutralization testing.
[b] Equivocal results

Prospective Samples Agreement Rates

Chronic Infection	Positive agreement with reference assay results = 100%	(1/1)
	95% CI = 2.5 to 100%	
	Negative agreement with reference assay results = NA	(0/0)
	95% CI = NA	
Recovery	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 100%	(2/2)
	95% CI = 15.8 to 100%	
HBV Vaccine Response	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 100%	(38/38)
	95% CI = 90.8 to 100%	
Past Infection	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 100%	(8/8)
	95% CI = 63.1 to 100%	
Not Previously Infected	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 98.9%	(86/87)
	95% CI = 93.8 to 99.9%	

10.1.2. Retrospective Samples

Retrospective studies were carried out at three clinical laboratories in the United States (California, Missouri, and Minnesota) and at DiaSorin (Italy) to assess the performance of the ETI-MAK-2 PLUS assay in detecting HBsAg. The study set included 650 frozen repository samples (the majority of which were purchased from commercial vendors) from the following populations:

- patients with chronic hepatitis B infection (HBsAg positive for greater than six months) - 111 frozen repository samples;
- patients with serologically diagnosed hepatitis B infection (acute, chronic, asymptomatic, convalescent, etc.) - 82 frozen repository samples;
- patients sent to the laboratory for hepatitis B testing - 100 frozen repository samples;
- a general hospital patient population - 357 frozen repository samples.

The specimens represented Midwestern (2%), Southeastern (25%), Western (13%), and Northeastern US (2%), outside of the US (1%), and unspecified (57%). The group was 44% (287/650) female, 42% (270/650) male, and 14% (93/650) unspecified. Approximately 13% (84/650) were Caucasian, 4% (27/650) were African American, < 1% (5/650) were Hispanic, < 1% (3/650) were Asian, and 82% (531/650) were unspecified. The ages ranged from 5 to 98 years old, with 131 specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-MAK-2 procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure with the exception of the HBsAg assay at two of the three sites. At these sites, the majority of specimens that were initially HBsAg-positive were repeated in duplicate, however the repeatedly reactive specimens were not confirmed by the licensed HBsAg confirmation assay at the two sites. Therefore, true HBsAg result was determined in one of three ways: 1) confirmed by reference assay neutralization during clinical trials, 2) based on a statement by the attending physician that HBsAg was positive for greater than 6 months, or 3) information provided by the vendor regarding confirmatory testing performed at their location or by the material source facility.

Results by Specimen Classification

After study completion all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 35 unique HBV marker patterns observed in the ETI-MAK-2 PLUS retrospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Acute Infection	+	+	+ or I*	+	-	-	52
	+	-	+ or I	+	+	-	4
	+	-	-	-	-	-	2
	+	+	-	-	-	-	2
Chronic Infection	+	-	-	+	+	-	82
	+	+	-	+	-	-	21
	+	-	-	+	- or I	-	23
	+	+	+	+	-	+	4
	+	+	- or I	+	-	+	2
	+	-	-	+	+	+	2
	+	+	-	+	+ or I	+	2
	+	+	+	+	+	+	1
	+	+	-	+	+	-	1
	+	-	-	+	-	+	1
Recovery	-	-	-	+	+ or I	+	40
	-	-	-	+	+	-	6
	-	-	+	+	+	-	2
	-	-	+ or I	+	+	+	2
Past Infection	-	- or I	-	+	-	+	12
	-	-	-	+	-	-	9
HBV Vaccine Response	-	-	-	-	-	+	20
Not Previously Infected with HBV	-	-	-	-	-	-	343
Uninterpretable	-	+ or I	-	-	-	-	13
	-	+	-	+	-	+	2
	-	+	-	+	+	+	1
	-	I	-	+	-	-	1

*I = indeterminate result

Based on the above classifications the ETI-MAK-2 PLUS HBsAg results for the retrospective samples were compared to a reference assay's HBsAg results as determined by the methods described above. The following tables show this comparison and percent agreement with 95% confidence intervals with the reference HBsAg results. The data are presented in three tables based on the means of determining true HBsAg results.

Retrospective Samples Comparison

True HBsAg Result Based on Statement from Physician

Reference Serology Classification	Reference HBsAg		TOTAL
	–	+	
	ETI-MAK-2 PLUS	ETI-MAK-2 PLUS	
	–	+	
Acute infection	0	26	26
Chronic infection	0	61	61
Recovery	1	0	1
Not previously infected	1	0	1
Grand Total	2	87	89

Retrospective Samples Agreement Rate

Acute Infection	Positive agreement with reference assay results = 100% (26/26) 95% CI = 86.8 to 100% Negative agreement with reference assay results = NA (0/0) 95% CI = NA
Chronic Infection	Positive agreement with reference assay results = 100% (61/61) 95% CI = 94.1 to 100% Negative agreement with reference assay results = NA (0/0) 95% CI = NA
Recovery	Positive agreement with reference assay results = NA (0/0) 95% CI = NA Negative agreement with reference assay results = 100% (1/1) 95% CI = 2.5 to 100%
Not Previously Infected	Positive agreement with reference assay results = NA (0/0) 95% CI = NA Negative agreement with reference assay results = 100% (1/1) 95% CI = 2.5 to 100.0%

Retrospective Samples Comparison

True HBsAg Result Based on Confirmation by Neutralization during Clinical Trials

Reference Serology Classification	Reference HbsAg				TOTAL
	–		+		
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS		
	–	+	–	+	
Acute infection	0	0	0	25	25
Chronic infection	0	0	1	47	48
Recovery	16	0	0	0	16
Past infection	6	0	0	0	6
HBV vaccine response	6	0	0	0	6
Not previously infected	110	3	0	0	113
Uninterpretable	5	2	0	0	7
Grand Total	143	5	1	72	221

Retrospective Samples Agreement Rate

Acute Infection	Positive agreement with reference assay results = 100%	(25/25)
	95% CI = 86.3 to 100%	
	Negative agreement with reference assay results = NA	(0/0)
	95% CI = NA	
Chronic Infection	Positive agreement with reference assay results = 97.9%	(47/48)
	95% CI = 88.9 to 99.9%	
	Negative agreement with reference assay results = NA	(0/0)
	95% CI = NA	
Recovery	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 100%	(16/16)
	95% CI = 79.4 to 100%	
HBV Vaccine Response	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 100%	(6/6)
	95% CI = 54.1 to 100.0%	
Past Infection	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 100%	(6/6)
	95% CI = 54.1 to 100%	
Not Previously Infected	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 97.3%	(110/113)
	95% CI = 92.4 to 99.4%	
Uninterpretable	Positive agreement with reference assay results = NA	(0/0)
	95% CI = NA	
	Negative agreement with reference assay results = 71.4%	(5/7)
	95% CI = 29.0 to 96.3%	

Retrospective Samples Comparison

True HBsAg Result Based on Vendor Information

Reference Serology Classification	Reference HbsAg				TOTAL
	–		+		
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS		
	–	+	+	E*	
Acute infection	0	0	6	0	6
Chronic infection	0	0	22	1	23
Recovery	33	0	0	0	33
Past infection	15	0	0	0	15
HBV vaccine response	13	1	0	0	14
Not previously infected	222	7	0	0	229
Uninterpretable	9	1	0	0	10
Grand Total	292	9	28	1	330

* Equivocal results

Retrospective Samples Agreement Rate

Acute Infection	Positive agreement with reference assay results =	100%	(6/6)
	95% CI = 54.1 to 100%		
	Negative agreement with reference assay results =	NA	(0/0)
	95% CI = NA		
Chronic Infection	Positive agreement with reference assay results =	100%	(22/22)
	95% CI = 84.6 to 100%		
	Negative agreement with reference assay results =	NA	(0/0)
	95% CI = NA		
Recovery	Positive agreement with reference assay results =	NA	(0/0)
	95% CI = NA		
	Negative agreement with reference assay results =	100%	(33/33)
	95% CI = 89.4 to 100%		
HBV Vaccine Response	Positive agreement with reference assay results =	NA	(0/0)
	95% CI = NA		
	Negative agreement with reference assay results =	92.9%	(13/14)
	95% CI = 66.1 to 99.8%		
Past Infection	Positive agreement with reference assay results =	NA	(0/0)
	95% CI = NA		
	Negative agreement with reference assay results =	100%	(15/15)
	95% CI = 78.2 to 100%		
Not Previously Infected	Positive agreement with reference assay results =	NA	(0/0)
	95% CI = NA		
	Negative agreement with reference assay results =	96.9%	(222/229)
	95% CI = 93.8 to 98.8%		
Uninterpretable	Positive agreement with reference assay results =	NA	(0/0)
	95% CI = NA		
	Negative agreement with reference assay results =	90.0%	(9/10)
	95% CI = 55.5 to 99.7%		

10.1.3. Samples from Pregnant Women:

Single samples collected from pregnant women (154 prospective samples and 410 retrospective samples) were tested with both DiaSorin and reference HBsAg assays. Positive results from the retrospective samples were confirmed by reference method neutralization at DiaSorin Italy after completion of the trials. Nonreactive results from the prospective samples were verified by testing for HBeAg, anti-HBe, total anti-HBc and IgM anti-HBc at the trial site. All nonreactive specimens were HBeAg and IgM anti-HBc nonreactive confirming the nonreactive HBsAg. The one positive sample from the prospective population was repeat tested for HBsAg and confirmed by reference method neutralization at the trial site. The table below compares the ETI-MAK-2 PLUS results with the HBsAg reference assay. The data in the table represent the number of specimens in each group. Equivocal results by the ETI-MAK-2 PLUS were repeated per the insert instructions, if sample volumes permitted and the repeat results used in the calculation.

Pregnant Women – Samples Comparison

Group	Reference HBsAg					Total
	–		+			
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS			
	–	+	–	+	E*	
Prospective Samples	154	0	0	0	0	154
Retrospective Samples	378	12	1	17	2	410
Total	532	12	1	17	2	564

* Equivocal results

Pregnant Women Prospective Samples Agreement Rate

Percent Positive Agreement =	0.% (0/0)	95% CI = NA
Percent Negative Agreement =	100.% (154/154)	95% CI = 97.6 to 100.

Pregnant Women Retrospective Samples Agreement Rate

Percent Positive Agreement =	85.0 (17/20)	95% CI = 62.1 to 96.8%
Percent Negative Agreement =	96.9% (378/390)	95% CI = 94.7 to 98.4

11. Conclusions Drawn from Studies

The study data demonstrates that acceptable performance is obtained with the DiaSorin ETI-MAK-2 PLUS assay when testing specimens collected in serum and plasma. There appeared to be no gender bias in the selection ratio or any difference in the safety and effectiveness based on gender. The DiaSorin assay shows acceptable within-run, between-run, between-day, site-to-site, and lot-to-lot reproducibility. The quality control procedures described in the package insert are appropriate to assure accurate assay performance. The data from this study provide reasonable assurance that the DiaSorin ETI-MAK-2 PLUS assay is safe and effective for its stated purpose when used as instructed in the package insert. The DiaSorin ETI-MAK-2 PLUS HBsAg assay can be stored up to 6 months weeks at 2-8°C.

Benefit/Risk

The submitted studies have shown that the DiaSorin ETI-MAK-2 PLUS assay, when compared to reference laboratory procedures, has a similar ability to detect the presence of HBsAg in specimens from individuals acutely and chronically infected with HBV. The rate of false positivity and false negativity are within acceptable limits compared to the reference assay. It has been shown that the device has no demonstrable cross-reactivity with viruses or organisms that may cause clinical hepatitis. Therefore, the devices should benefit the physician in the diagnosis of HBV associated and non-associated hepatitis. The devices will also aid in the identification of pregnant women infected with HBV. Therefore it is reasonable to conclude that the benefits of use of the device for the target population outweigh the risk of illness or injury when used as indicated when used accordance with the directions for use.

12. Panel Recommendations

The Microbiology Advisory Panel met on January 20, 2000, to consider the safety and effectiveness of the ETI-MAK-2 PLUS assay. The Panel recommended approval subject to the following conditions.

- Conduct additional studies for the immunity claim by testing individuals immediately after receiving the complete series of three vaccinations with the hepatitis B virus vaccines and three to nine months later.
- Provide more data on acute/chronic infections in high-risk populations such as those individuals that are infected with HIV, sexually transmitted diseases, and those patients that are immunosuppressed.
- Collect more data on patients meeting the standard definition for chronicity, i.e., > 6 months of infection.

.

13. CDRH Decision

CDRH concurred with the Panel's recommendation. DiaSorin Inc. has provided some additional data to address some of the Panel's issues and those issues not fully resolved were addressed with labeling restrictions and the requirement of postapproval studies. The two postapproval studies were:

- Within 6 months of this approval, DiaSorin should submit a reproducibility study for the Biochem Immunosystems Labotech/ETI-Lab automated instrument.

- To address the concerns made by the Panel regarding the retrospective nature of the clinical studies, within 2 years of this approval, DiaSorin should submit the results of an additional prospective clinical study. We suggest that this study involve individuals that may be considered representative of an U.S. population, i.e., similar prevalence of HBV disease and serotypes.

The applicant's manufacturing facility was found to be in compliance with the Quality Systems Regulation (21 CFR 820).

CDRH issued an approval order on March 30, 2001.

14. Approval Specifications

Directions for use: See Labeling

Conditions of Approval: CDRH Approval of this PMA is subject to full compliance with the conditions described in the approval order.